migration of tumor cells to neighbouring tissue as well as in metastasis to distant sites in the body via newly formed blood vessels (angiogenesis).
We investigated CXCR4- SDF1α mediated chemotaxis in mouse fibroblasts in an integrated approach from the tissue to the single-molecule level. First, we characterized cellular migratory potential upon stimulation with SDF1α in wound healing assays applying phase contrast microscopy. We find that transiently transfected cells expressing CXCR4 double their migration speed in comparison to wild type 3T3 cells. Second, we applied single-molecule fluorescence microscopy to study the mobility of the G protein-coupled receptor CXCR4-eYFP in resting cells and upon stimulation with SDF1α. Two fractions of receptors prior to stimulation were identified: half of the receptors were immobile while the other half exhibited free diffusion with D ~ 0.3 $\mu m^2/s$ on short timescales (up to 100 ms). At longer timescales receptors showed confined diffusion within micrometer domains. Global stimulation with SDF1α switched a subset of the receptors from the immobile to the mobile fraction. We predict that the impact of a SDF1α gradient might lead to asymmetric receptor diffusion and subsequently polarized cell behaviour as seen in the wound healing assays.

114-Plat
Quantitative Description of Signaling Downstream of Gq-Coupled Receptors: Similarities and Differences in the Responses of IP3, Calcium, DAG, PKC, and PIP2
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Gq-coupled plasma membrane receptors modulate cellular functions by activating phospholipase C (PLC), which hydrolyses the membrane lipid phosphatidylinositol (4,5)-bisphosphate (PIP_2) into the second messengers inositoltrisphosphate (IP_3) and diacylgycerol (DAG). To better understand the mechanisms that govern these partially independent signals we monitored in single, living tsA-201 cells levels of PIP_2, IP_3, calcium, DAG, and PKC by optical probes and current. We compared (i) activation of (low-abundance) endogenous purinergic receptors and overexpressed M_1 muscarinic receptors, and (ii) different concentrations of the muscarinic agonist oxotremorine-M (oxo-M). Whereas the peak responses from reporters of IP_3 (LIBRAvIII) and DAG (C1 domains of PKCγ) scale with abundance of receptor or agonist, downstream production of calcium (Fura4F) and PKC activation (CKAR) do not. Amplitude and duration of calcium signals elicited by 100 μM UTP, 10 nM oxo-M, or 10 μM oxo-M are almost identical. The only difference is a shorter latency with 10 μM oxo-M. These data suggest that a relatively low amount of IP_3 is required for calcium release. This interpretation is supported by the finding that a full-size calcium response can still be elicited after PIP_2 is depleted by recruiting a PI 5-phosphatase to the plasma membrane (by rapamycin-induced dimerization). Duration and late recovery time courses are different between IP_3 (duration=68 s; τ_{off} =55 s) and calcium (duration=110 s; τ_{off}=34 s), suggesting that once a threshold of IP_3 is reached, the calcium signal unfolds. Therefore we conclude that the IP_3 requirement for calcium release must be low. The time point and IP_3 level (from LIBRAvIII) at which the calcium response starts can provide an estimate of this IP_3 threshold. Supported by NIH grants NS08174 & GM83913 and the HFSP.

115-Plat
Vitamin A as an Activator and Sensitizing Chromophore for Rhodopsin
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Absorption of light by rhodopsin isomerizes its 11-cis retinal chromophore to the all-trans conformation. The rhodopsin then activates a biochemical cascade that produces an electrical response by the photoreceptor. Eventually, all-trans retinal dissociates from the opsin and is reduced to vitamin A. The truncated retinal analog, beta-ionone, can pharmacologically activate some types of visual pigment, mimicking the effects of light. Beta-ionone is not normally found in the retina, however, vitamin A is present within the photoreceptor and can reach millimolar concentrations after exposure to bright light. Can vitamin A activate rhodopsin? In suction electrode recordings from isolated green-sensitive rods of salamander, exogenous vitamin A decreased circulating current and flash sensitivity, and accelerated flash response kinetics, changes that can also be seen during exposure to background light. Microspectrophotometric measurements showed that vitamin A accumulated in the outer segments, and an in vitro binding assay confirmed the binding of vitamin A to rhodopsin. These results suggested that vitamin A activated rhodopsin. In addition, suction electrode recordings showed that vitamin A improved the relative sensitivity of rods to UV light and in an in vitro bleaching assay, vitamin A enhanced the bleaching of rhodopsin by UV light. Presumably, both effects involved Forster resonance energy transfer (FRET) from vitamin A to the 11-cis chromophore of rhodopsin. Next, we tested whether FRET could be induced by endogenous vitamin A. After bleaching a large fraction of the rhodopsin in green-sensitive rods to generate vitamin A, relative sensitivity to UV wavelengths did indeed increase. Therefore, vitamin A can bind rhodopsin, activate it and also serve as a sensitizing chromophore.

PLATFORM I: Molecular Mechanics & Force Spectroscopy I

116-Plat
Viscoelasticity of Globular Proteins Measured from the AC Susceptibility
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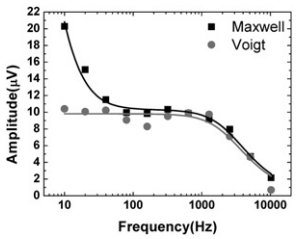
We introduce a new method to measure the elasticity and internal viscosity of nanometer size biological molecules such as globular proteins. Gold nanoparticles, tethered to a gold surface by the protein, are driven by an AC electric field while their displacement is synchronously detected by evanescent wave scattering, yielding the mechanical response function of the macromolecular sample in the frequency domain. We apply the method to measure the both the elastic constant and internal viscosity of proteins.



117-Plat
Designed Biomaterials to Mimic the Passive Elastic Properties of Muscles
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The passive elasticity of muscle is largely governed by the I-band part of the giant muscle protein titin, a complex molecular spring composed of a series of individually folded immunoglobulin-like domains as well as largely unstructured unique sequences. These mechanical elements have distinct mechanical properties, and when combined, they provide the desired passive elastic properties of muscle, which are a unique combination of strength, extensibility and resilience. Single-molecule atomic force microscopy (AFM) studies demonstrated that the macroscopic behaviour of titin in intact myofibrils can be reconstituted by combining the mechanical properties of these mechanical elements measured at the single-molecule level. Here we report artificial elastomeric proteins that mimic the molecular architecture of titin through the combination of well-characterized protein domains GB1 and resilin. We show that these artificial elastomeric proteins can be photochemically crosslinked and cast into solid biomaterials. These biomaterials behave as rubber-like materials showing high resilience at low strain and as shock-absorber-like materials at high strain by effectively dissipating energy. These properties are comparable to the passive elastic properties of muscles within the physiological range of sarcomere length and so these materials represent a new muscle-mimetic biomaterial. The mechanical properties of these biomaterials can be fine-tuned by adjusting the composition of the elastomeric proteins, providing the opportunity to develop biomaterials that are mimetic of different types of muscles. We anticipate that these biomaterials will find applications in tissue engineering as scaffold and matrix for artificial muscles.

118-Plat
Influenza Virus Adhesion to Living Cells Measured by Single Virus Force Spectroscopy (SVFS) and Force Probe MD Simulation
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Influenza virus belongs to a wide range of viruses that are enclosed in a lipid envelope. The major spike protein of the viral envelope hemagglutinin (HA) binds sialic acid (SA) residues of glycoproteins on the plasma membrane of